Antibacterial Activity of Metergoline Analogues: Revisiting the Ergot Alkaloid Scaffold for Antibiotic Discovery

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ABSTRACT: Metergoline is a semisynthetic ergot alkaloid identified recently as an inhibitor of the Gram-negative intracellular pathogen *Salmonella* Typhimurium (*S.* Tm). With the previously unknown antibacterial activity of metergoline, we explored structure−activity relationships (SARs) with a series of carbamate, urea, sulfonamide, amine, and amide analogues. Cinnamide and arylacrylamide derivatives show improved potency relative to metergoline against Gram-positive bacteria, and pyridine derivative 38 is also effective against methicillin-resistant *Staphylococcus aureus* (MRSA) in a murine skin infection model. Arylacrylamide analogues of metergoline show modest activity against wild-type (WT) Gram-negative bacteria but are more active against strains of efflux-deficient *S.* Tm and hyperpermeable *Escherichia coli*. The potencies against WT strains of *E. coli*, *Acinetobacter baumannii*, and *Burkholderia cenocepacia* are also improved considerably (up to >128-fold) with the outer-membrane permeabilizer SPR741, suggesting that the ergot scaffold represents a new lead for the development of new antibiotics.

✓ Improved potency vs S. Tm, S. aureus, B. subtilis, M. smegmatis
✓ Effective vs MRSA in a murine skin infection model
✓ Synergy with SPR741 vs Gram-negative bacteria

KEYWORDS: Antibacterial, metergoline, ergot alkaloid, Gram-positive, Gram-negative
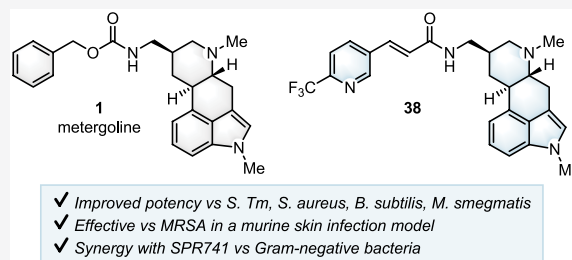
Antimicrobial resistance in Gram-positive and Gram-negative bacteria is a threat to global public health.[1,2] Multidrug-resistant strains of pathogenic bacteria can be resistant to all available antibiotics, and untreatable infections have become increasingly common in the clinic.[3] Gram-negative bacteria are especially problematic because of their ability to acquire multiple resistance elements, and their outer membrane (OM) is a formidable barrier that prevents the entry of most antibiotics.[4−6] Indeed, there is a pressing need to develop new antibiotics and adjuvants with new scaffolds and mechanisms of action.[7−9]

Salmonella enterica serovar Typhimurium (*Salmonella* Typhimurium, *S.* Tm) is on the WHO list of priority pathogens[10] as a leading cause of gastroenteritis worldwide[11] and classified as a serious antibiotic resistance threat by the U.S. Centers for Disease Control and Prevention.[1] *S.* Tm is a Gram-negative intracellular pathogen that evades the host's innate immune response by replicating within host phagocytes.[12,13] Intracellular environments differ considerably from nutrient-rich growth media.[13] Genes that are nonessential in rich media can become essential *in vivo* and therefore represent new antimicrobial targets.[14]

We recently developed a phenotypic high-throughput screen for inhibitors of *Salmonella* growth within macrophages and identified metergoline (**1**) as a hit (Figure 1).[15] Although metergoline showed no activity against *S.* Tm *in vitro* in nutrient-rich MHB medium, it was still able to effectively inhibit *S.* Tm
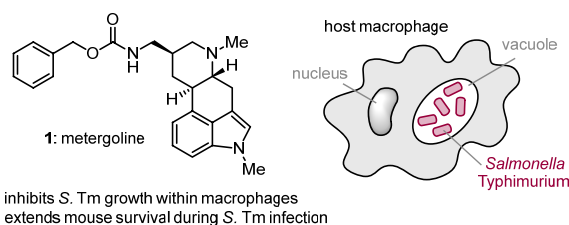
Figure 1. Metergoline inhibits the growth of the Gram-negative intracellular pathogen *Salmonella* Typhimurium (*S.* Tm).[15]

growth within macrophages. Remarkably, metergoline also extended the survival of mice during systemic *S.* Tm infection.[15] Metergoline shows minimal structural resemblance to clinically used antibacterials and could not have been identified in conventional screens that use nutrient-rich growth media.

Metergoline is a semisynthetic ergot alkaloid that has been used clinically (as Liserdol) for the treatment of hyper-prolactinemia and investigated for other disorders.[16] Ergot
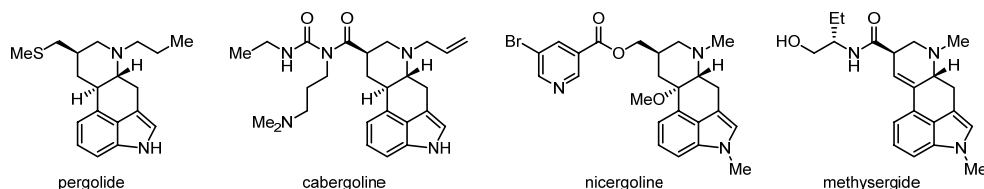
A *Semisynthetic ergot alkaloids*

pergolide   cabergoline   nicergoline   methysergide
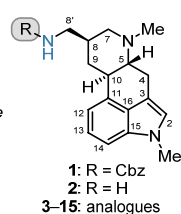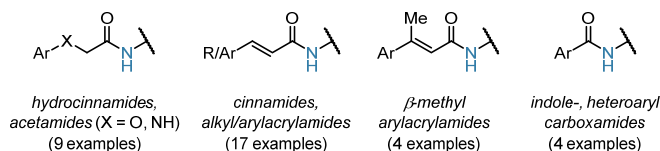
B *Ellis et al. (2019)[15]*

carbamate, sulfonamide, urea, amine, amide analogues of metergoline (14 examples)
● *Preliminary SAR vs S. Tm, MRSA*

1: R = Cbz
2: R = H
3–15: analogues

C *This work:* ● Expanded series of amide- and acrylamide analogues of metergoline
● Expanded SAR vs Gram-positive bacteria, Gram-negative bacteria, and mycobacteria

hydrocinnamides, acetamides (X = O, NH) (9 examples)

cinnamides, alkyl/arylacrylamides (17 examples)

β-methyl arylacrylamides (4 examples)

indole-, heteroaryl carboxamides (4 examples)

Figure 2. Metergoline analogues and related ergot alkaloids. (A) A series of semisynthetic ergot alkaloids used clinically. (B) Metergoline analogues prepared previously[15] for preliminary antibacterial structure−activity relationship (SAR) studies. (C) The expanded series of metergoline analogues evaluated in the present study.

Table 1. Antibacterial Activities of Ergot Alkaloids and Metergoline Analogues[a]

Compound	S. Tm SL1344 growth in macrophages[b]	S. Tm SL1344 WT MHB[g]	S. Tm SL1344 WT LPM[h]	S. Tm ΔtolC MHB	E. coli MG1655 WT[c] MHB	E. coli MG1655 WT[c] MOPS[i]	E. coli MG1655 ΔtolC-pore[d] MHB	E. coli MG1655 ΔtolC-pore[d] MOPS	MRSA WT[e] MHB	B. subtilis 168[f] MHB
pergolide	0.93	>200	>200	>200	>200	>200	>200	>200	>200	>200
cabergoline	0.75	>200	>200	>200	>200	>200	>200	128	>200	>200
nicergoline	0.59	>200	>200	128	>200	>200	128	200	200	200
methysergide	1.01	>200	>200	>200	>200	>200	>200	128	128	>200
1: R = Cbz	0.08	>200	200	32	200	128	32	8.32	32	32
2: R = H	0.39	>200	>200	>200	>200	128	128	128	>200	200
3: R = Boc	0.23	>200	>200	200	>200	>200	200	200	200	200
4: R = CO$_2$Et	0.85	>200	>200	>200	>200	>200	>200	>200	>200	>200
5: R = Ac	0.84	>200	>200	>200	>200	>200	>200	200	>200	>200
6: R = Bz	0.65	>200	>200	200	>200	>200	200	128	>200	>200
7: R = COCH$_2$Ph	0.86	>200	>200	200	>200	>200	200	64	>200	200
16: X = O	nt	>256	>256	256	>256	>256	128	64	256	128
17: X = NH	nt	>256	>256	256	>256	>256	128	64	256	256
8: YZ = NHCO	0.49	>200	>200	128	>200	>200	128	32	128	128
9: YZ = CH$_2$SO$_2$	0.08	>200	200	64	>200	>200	32	32	64	32
10: YZ = CH$_2$CH$_2$	0.58	>200	>200	64	128	64	64	16	64	16
11: R = H	0.87	>200	>200	200	>200	>200	128	64	128	200
12: R = NHBoc	nt[j]	>200	>200	64	>200	>200	128	16	64	64
13: R = NH$_2$	0.56	>200	200	200	>200	128	200	32	64	128
14: R = H	0.07	>200	128	16	>200	>200	8.32	8.32	16	64
15: R = 4-Cl	0.08	>200	128	16	>200	>200	8.32	8.32	2.56	32

[a]Minimum inhibitory concentrations (MICs) are shown in μg/mL. Assays were run in duplicate in 384-well plate format, except for **16** and **17**, which were run in 96-well plate format. [b]Growth of wild-type *Salmonella* Typhimurium SL1344 in RAW264.7 macrophages in the presence of 8 μg/mL compound, relative to a DMSO-treated control (data from Ellis et al.[15]). [c]*Escherichia coli* K-12 strain MG1655. [d]A hyperpermeable strain of *E. coli* that lacks TolC and expresses a truncated (pore-only) form of FhuA. [e]Methicillin-resistant *Staphylococcus aureus* strain USA300. [f]*Bacillus subtilis* strain 168. [g]MHB = Mueller−Hinton broth. [h]LPM = acidic, low-phosphate, low-magnesium medium. [i]MOPS = Neidhardt minimal medium for enterobacteria. [j]nt = not tested.

alkaloids are fungal natural products that are ligands for serotonin and dopamine receptors and have a variety of different pharmacological profiles, clinical applications, and antitumor, insecticidal, and antiparasitic activities.[17−20] Metergoline itself has been investigated as an antifungal agent[21,22] and antimalarial,[23] but its antibacterial properties were unknown prior to our recent report.[15] Some clavine-type alkaloids have shown moderate antibacterial activity (Figure S1),[24] but

structure−activity relationships (SARs) and their antibacterial mechanisms of action remain unknown.

The antibacterial activity of metergoline was unusual because it was inactive against *S.* Tm grown in MHB *in vitro* but sufficiently potent *in vivo* to inhibit intracellular *S.* Tm growth and extend mouse survival during a lethal systemic infection. Further investigations revealed that the integrity of the *S.* Tm

Table 2. Antibacterial Activities of Amide Analogues of Metergoline[a]

Metergoline analogue	S. Tm SL1344 WT		S. Tm ΔtolC	E. coli MG1655 WT		E. coli MG1655 ΔtolC-pore		MRSA WT	B. subtilis 168
	MHB	LPM	MHB	MHB	MOPS	MHB	MOPS	MHB	MHB
1: metergoline	>200	200	32	200	128	32	8.32	32	32
18: R = 2-F	>200	>200	200	>200	>200	200	128	200	>200
19: R = 4-F	>200	>200	128	>200	>200	64	16	128	128
20: R = 3-Cl	>200	>200	128	>200	>200	128	32	200	200
21: R = 4-Cl	>200	128	32	>200	>200	32	8.32	16	64
22: R = 4-Br	>200	200	64	>200	>200	32	10.24	10.24	64
23: R = 2-OMe	>200	>200	200	>200	>200	200	128	128	>200
24: R = 3,4-(OMe)₂	>200	>200	>200	>200	>200	>200	>200	>200	>200
25: R = 4-Me	>200	200	16	>200	>200	10.24	16	5.12	64
26: R = 4-F	>200	128	32	>200	>200	16	5.12	10.24	32
27: R = 4-Br	>200	>200	16	>200	>200	10.24	8.32	5.12	64
28: R = 3-CF₃	>200	32	3.84	>200	>200	2.56	3.84	3.84	5.12
29: R = 4-CF₃	>200	32	>200	>200	>200	>200	32	2.56	32
30: R = 4-NMe₂	>200	>200	128	>200	>200	200	64	32	>200
31: R = 4-OMe	>200	>200	32	>200	>200	32	16	10.24	64
32: R = 2,4-(CF₃)₂	>200	32	16	>200	>200	16	5.12	8.32	8.32
33: R = 2-F-4-CF₃	>200	>200	>200	>200	>200	>200	>200	5.12	64
34: R = 4-Cl-3-CF₃	>200	16	>200	>200	>200	128	>200	64	64
35: n = 1	>200	128	16	>200	>200	8.32	16	16	64
36: n = 2	>200	>200	8.32	>200	>200	5.12	10.24	8.32	64
37: X = N, Y = CH	>200	>200	200	>200	>200	200	64	>200	>200
38: X = CCF₃, Y = N	>200	128	8.32	>200	>200	5.12	8.32	1.28	32
39: R, X, Y = H, N, NH	>200	>200	64	>200	>200	32	16	>200	200
40: R, X, Y = Br, CH, NH	>200	>200	32	>200	>200	32	3.84	16	16
41: R, X, Y = H, CH, S	>200	>200	10.24	>200	>200	5.12	8.32	16	16
42: R = H	>200	>200	8.32	>200	>200	5.12	5.12	32	32
43: R = 4-Cl	>200	32	10.24	>200	>200	10.24	5.12	8.32	8.32
44: R = 4-Br	>200	32	8.32	>200	>200	8.32	3.84	8.32	8.32
45: R = 4-SO₂Me	>200	>200	>200	>200	>200	128	64	200	>200
46: R = H, X-Y = C-CH	>200	64	32	>200	>200	16	5.12	32	32
47: R = Br, X-Y = C-CH	>200	64	32	>200	>200	64	8.32	16	32
48: R = H, X-Y = C-N	>200	>128	32	>200	>200	16	16	>200	128
49: R = H, X-Y = N-CH	>200	>200	>200	>200	>200	>200	128	>200	>200
50: trans-(RS,RS)	>200	200	64	>200	>200	32	10.24	64	64
51	>200	>200	>200	>200	>200	>200	128	>200	>200

[a]Minimum inhibitory concentrations (MICs) are shown in μg/mL. Assays were run in duplicate in 384-well plate format.

OM is compromised within macrophages, thereby making *S.* Tm more permeable toward metergoline.[15]

Interestingly, other related semisynthetic ergot alkaloids with only subtle structural differences at the 1-, 6-, and 10-positions of the ergot core (Figure 2A) were inactive against strains of *S.* Tm and methicillin-resistant *Staphylococcus aureus* (MRSA) (Table 1). These preliminary SARs indicated that the benzyl carbamate moiety of metergoline is important for antibacterial activity. We synthesized a series of carbamate, sulfonamide, urea, amine, and amide analogues of metergoline (**2−15**; Figure 2B) and found that carbamate, urea, and amine analogues **2**, **3**, **8**, **10**, and **13** showed moderate activities versus *S.* Tm and MRSA, whereas the sulfonamide and cinnamide analogues **9** and **14** had

activities comparable to that of metergoline.[15] *p*-Chlorocinnamide **15**, however, showed improved potency against *S.* Tm (2-fold) and MRSA (>12-fold) compared with metergoline, suggesting that additional modifications of the 8′ substituent could further improve the potency and spectrum of activity.

In this work, we report the synthesis of an expanded series of amide analogues of metergoline and their activities against additional strains of Gram-positive, Gram-negative, and mycobacteria (Figure 2C). Some arylacrylamide analogues were highly potent against MRSA *in vitro*, and one was also efficacious *in vivo* in a skin infection model. Metergoline analogues showed poor activity against wild-type (WT) Gram-negative bacteria but were substantially more potent in

combination with the OM permeabilizer SPR741[25] or with genetic disruption of the cell envelope. Metergoline analogues therefore represent a promising new scaffold for the development of new antibiotics with potential broad-spectrum activity.

RESULTS AND DISCUSSION

Bacterial Strains Evaluated. We evaluated metergoline and structural analogues against WT *S.* Tm in MHB medium and also in an acidic low-phosphate, low-magnesium medium (LPM) that was developed to mimic the intracellular environment of the *Salmonella*-containing vacuole (SCV).[26] Growth of *S.* Tm is more sensitive to metergoline and other antibiotics in LPM compared with growth in MHB, and LPM provides a validated approximation of the intracellular environment in which *S.* Tm propagates during an infection.[15] Since metergoline is also a substrate for the AcrAB—TolC efflux pump and we sought to gain further insights into the SARs among metergoline and analogues, we also used an efflux-deficient ($\Delta tolC$) strain of *S.* Tm. In order to probe the breadth of antibiotic activity, we also assayed compounds against *Escherichia coli*, MRSA strain USA300, and *Bacillus subtilis* strain 168. Assays with *E. coli* used nutrient-rich MHB and nutrient-poor Neidhardt minimal medium for enterobacteria (MOPS). We also constructed a hyperpermeable strain of *E. coli* MG1655 that is efflux-deficient ($\Delta tolC$) and also constitutively expresses additional pore proteins. The pore used here was a known truncated version of the siderophore receptor FhuA[27,28] that lacks its "gating loop" but retains its hollow β-barrel domain and effectively functions as an open channel.

Activities of Ergot Alkaloids. As with *S.* Tm and MRSA, metergoline was also the most active of the ergot alkaloids against strains of *E. coli* and *B. subtilis* (Table 1). Although pergolide, cabergoline, nicergoline, and methysergide were inactive against all strains, metergoline showed moderate potency against *B. subtilis* and *E. coli* $\Delta tolC$-pore and improved activity versus *E. coli* $\Delta tolC$-pore in nutrient-limited MOPS.

Analogues of the Benzyl Carbamate. For carbamate, amide, urea, amine, and amide analogues of metergoline **2−15**, the antibacterial activities against strains of *S.* Tm and MRSA were generally comparable to those against strains of *E. coli* and *B. subtilis*, respectively. Carbamate and amide analogues **3−7**, **16**, and **17** were inactive, but sulfonamide **9** and cinnamides **14** and **15** were among the most potent analogues. Sulfonamide **9** showed activity comparable to that of metergoline against most strains, but cinnamoyl derivatives **14** and **15** were the only analogues to show improvements against *S.* Tm $\Delta tolC$, MRSA, and *E. coli* $\Delta tolC$-pore. The activity of cinnamide **15** was especially interesting because a single *p*-Cl substitution improved the potency >6-fold against MRSA, compared with the unsubstituted cinnamide **14**. This SAR, together with the activity against both Gram-positive and Gram-negative bacteria, encouraged further explorations of 8′-side-chain modifications and different substitutions on the aromatic ring.

Cinnamoyl and Hydrocinnamoyl Derivatives. The large differences in activities between amides **11** and **14** may be a reflection of differences in flexibility. Highly flexible molecules often suffer higher entropic penalties for target binding or for membrane permeability compared with more conformationally restricted molecules with fewer rotatable bonds.[29−31] In order to gain further insights into the SAR, we synthesized and evaluated a series of hydrocinnamoyl (3-phenylpropanoyl)- and cinnamoyl analogues **18−34** (Table 2). Many of the amides were prepared from reactions of amine **2** with acid chlorides or from

commercially available carboxylic acids using the standard peptide coupling reagent *N*-ethyl-*N*′-dimethylaminopropyl carbodiimide (EDC) (Figure S2). Arylacrylic acid precursors were prepared from the corresponding benzaldehydes in two steps *via* Horner—Wadsworth—Emmons (HWE) olefination followed by alkaline hydrolysis.

Methoxy-substituted hydrocinnamoyl derivatives **23** and **24** were inactive, but halogenated analogues showed improved potency, especially against *E. coli* $\Delta tolC$-pore and MRSA, with *p*-Cl and *p*-Br analogues **21** and **22** being most active. Cinnamoyl derivatives **14**, **15**, **26**, and **27** were superior to their saturated counterparts **11**, **21**, **19**, and **22**, respectively. Among cinnamides, the most potent against MRSA were the *p*-Cl and *p*-CF$_3$ analogues **15** and **29**, each with an MIC of 2.56 μg/mL. The 3-CF$_3$ derivative **28** showed comparable potency against MRSA (3.84 μg/mL) but also showed improved activity against *S.* Tm in LPM, *S.* Tm $\Delta tolC$, *E. coli* $\Delta tolC$-pore, and *B. subtilis*. Multiple substituents were tolerated in some cases (*e.g.*, **32** and **33**), but the combination of two individually beneficial substituents, 4-Cl and 3-CF$_3$ (as in **34**), was detrimental to the activity, suggesting that additional steric bulk can hinder the activity. As exceptions to the trend among other analogues, **29** and **34** were more potent against *S.* Tm in LPM than *S.* Tm $\Delta tolC$ in MHB.

3-Alkyl- and 3-Arylacrylamide Analogues. Saturated bioisosteres of benzene are often used to improve potency and metabolic stability,[32] and increasing the fraction of sp^3-hybridized carbons (Fsp^3) can also improve the solubility.[33,34] Interestingly, cyclopentyl and cyclohexyl derivatives **35** and **36** showed activities comparable to that of cinnamide **14** against all strains tested.

Polarity, solubility, and log D can also be improved by substituting benzene rings with nitrogen-containing heterocycles.[35] 4-Pyridyl analogue **37** was inactive, but 4-CF$_3$-3-pyridyl derivative **38** showed improved activity against MRSA (1.28 μg/mL), *S.* Tm $\Delta tolC$, and *E. coli* $\Delta tolC$-pore compared with the *p*-Cl and *p*-CF$_3$ cinnamides **15** and **29**. Azaindole analogue **39** showed diminished activity against most strains, but 5-bromoindole and benzothiophene derivatives **40** and **41** showed moderate potency against *S.* Tm $\Delta tolC$ and *E. coli* $\Delta tolC$-pore.

Sterically Hindered and Conformationally Restricted Cinnamide Analogues. We continued developing metergoline SARs by exploring substitutions at the α- and β-positions of the cinnamide. Unsubstituted acrylamides are weakly electrophilic functional groups that have been used in the design of targeted covalent inhibitors,[36] but recent control experiments demonstrated that acrylamides have low reactivity toward nucleophiles (*e.g.*, glutathione),[37] especially with substituents at the β-position.[38,39] However, we sought to further limit any potential off-target reactivity by designing β-methyl derivatives **42−45**, heterocyclic analogues **46−49**, and cyclopropanes **50** that would be sterically hindered or nonelectrophilic analogues and may also have different conformational preferences that are beneficial for activity.

Among the fused heterocycle series, indole **46** showed activity comparable to that of cinnamide **14**, but bromoindole **47**, benzimidazole **48**, and imidazo[1,2-*a*]pyridine **49** were less potent. Cyclopropane **50**, which was tested as a mixture of two *trans* diastereomers, showed moderate activity against *E. coli* $\Delta tolC$-pore but was inactive against the other strains. Interestingly, β-methyl derivatives **42−44** showed improved activity against *S.* Tm, *E. coli*, and *B. subtilis* strains compared with **14**, **15**, and **27**, respectively, but were slightly less active

Table 3. Antibacterial Activities of Metergoline Analogues in the Presence and Absence of SPR741[a]

Strain	1 0 µg/mL SPR741	1 10 µg/mL SPR741	15 0 µg/mL SPR741	15 10 µg/mL SPR741	28 0 µg/mL SPR741	28 10 µg/mL SPR741	38 0 µg/mL SPR741	38 10 µg/mL SPR741	44 0 µg/mL SPR741	44 10 µg/mL SPR741
Staphylococcus aureus (MRSA)[b]	32	nt[e]	2	nt	8	nt	1	nt	8	nt
Staphylococcus aureus (MSSA)[c]	64	nt	1	nt	4	nt	1	nt	8	nt
Mycobacterium smegmatis	32	nt	0.25	nt	0.5	nt	2	nt	4	nt
Escherichia coli WT MG1655	>128	32	>128	4	>128	1	>128	8	>128	8
Escherichia coli ΔtolC + pore	32	32	8	4	1	0.5	4	4	8	4
Escherichia coli EHEC[d]	>128	64	>128	8	>128	2	>128	8	>128	8
Acinetobacter baumannii C0015	>128	32	>128	8	>128	4	>128	>128	>128	4
Burkholderia cenocepacia	>128	32	>128	4	>128	1	>128	4	>128	4
Klebsiella pneumoniae ATCC43816	>128	64	>128	16	>128	>128	>128	>128	>128	64
Pseudomonas aeruginosa PA01	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128

[a]MICs are shown in µg/mL. Assays were run in duplicate in 96-well plate format with MHB for each strain. [b]Methicillin-resistant *S. aureus* strain USA300. [c]Methicillin-susceptible *S. aureus* (MSSA) Newman strain. [d]EHEC = enterohemorrhagic *E. coli*. [e]nt = not tested.

against MRSA. Methyl sulfone **45**, which is sterically bulkier than halogenated analogues **43** and **44**, showed poor activity. Overall, the comparable potencies of cinnamides **14**, **15**, and **27** and their sterically more hindered β-methyl analogues **42−44** implies that the electrophilic character of the cinnamide moiety is not a contributing factor for activity. Furthermore, a simplified analogue (**51**) that retains the cinnamide but lacks the ergoline core was inactive against all strains.

Metergoline Analogues versus *S. aureus* and *M. smegmatis*. Among the most potent and broad-spectrum metergoline analogues against the strains of *S.* Tm, *E. coli*, MRSA, and *B. subtilis* were compounds **15**, **28**, **38**, and **44**. To further probe the extent of their antibiotic activities, we evaluated these compounds against additional strains of Gram-positive bacteria, mycobacteria, and Gram-negative bacteria (Table 3).
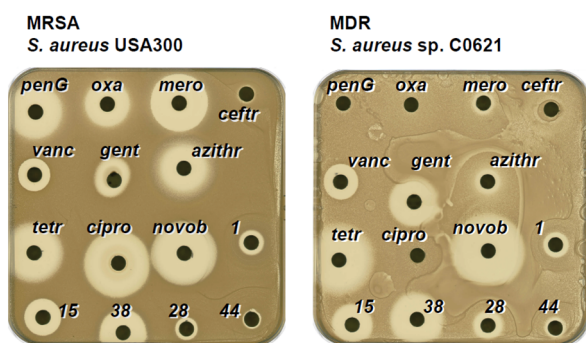
The metergoline analogues showed comparable potencies against methicillin-resistant and methicillin-susceptible strains of *S. aureus*, with MIC values as low as 1 µg/mL. Interestingly, the compounds were also active against a multidrug-resistant (MDR) clinical isolate of *S. aureus* (Figure 3). Disc diffusion assays show that *S. aureus* strain C0621 has reduced susceptibility to penicillin G, oxacillin, meropenem, ceftriaxone, azithromycin, and ciprofloxacin, compared with MRSA USA300, but the metergoline analogues remain active. These data indicate that metergoline analogues are unaffected by


Figure 3. Disc diffusion assays and the susceptibility of *S. aureus* strains USA300 and C0621 toward metergoline analogues and the clinically used antibiotics penicillin G, oxacillin, meropenem, ceftriaxone, vancomycin, gentamycin, azithromycin, tetracycline, ciprofloxacin, and novobiocin.

existing resistance to β-lactams, macrolides, and quinolones and suggest that they could have clinical usefulness against infections that are difficult to treat with standard antibiotics.

Encouraged by the potency of the metergoline analogues against different strains of *S. aureus*, we tested the efficacy of 4-CF₃-3-pyridyl derivative **38** against MRSA in a murine skin infection model (Figure 4). A superficial skin infection was
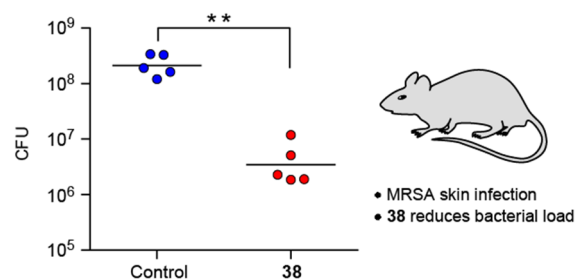
Figure 4. Metergoline analogue **38** is efficacious against MRSA (*S. aureus* USA300) in a murine skin infection model. Superficial skin infections were treated with ointment supplemented with either vehicle (2.5% DMSO, blue circles) or compound **38** (0.1% w/v, red circles). Groups of mice (n = 5) were treated at 1, 4, 8, 12, and 20 h postinfection. Bacterial load in wounded tissue was measured after the experimental end point of 24 h postinfection. Horizontal lines represent the geometric means of the bacterial loads for the two treatment groups. **, $P < 0.01$.

established by partial removal of the epidermal layer, followed by application of ~10⁶ colony forming units (CFU) of MRSA. Wounds were treated five times over 24 h with an ointment containing either the DMSO vehicle or compound **38**. The cohort treated with **38** showed a ~2 log₁₀ reduction (*i.e.*, ~98%) in CFU in wounded tissue compared with the control group.

Metergoline analogues **15**, **28**, **38**, and **44** also showed potent activity against *Mycobacterium smegmatis*, with MIC values of 0.25−4 µg/mL (Table 3). These results are encouraging because mycobacterial cell envelopes, which have topologies and compositions distinct from those of Gram-positive and Gram-negative bacteria, represent a challenging barrier for anti-mycobacterial drugs.[40] Mycobacteria (*e.g.*, *Mycobacterium tuberculosis*) are also intracellular pathogens, and mycobacterial persisters are known to be particularly unresponsive to antibiotic treatment.

Metergoline Analogues versus Gram-Negative Bacteria. Compounds **15**, **28**, **38**, and **44** were also evaluated
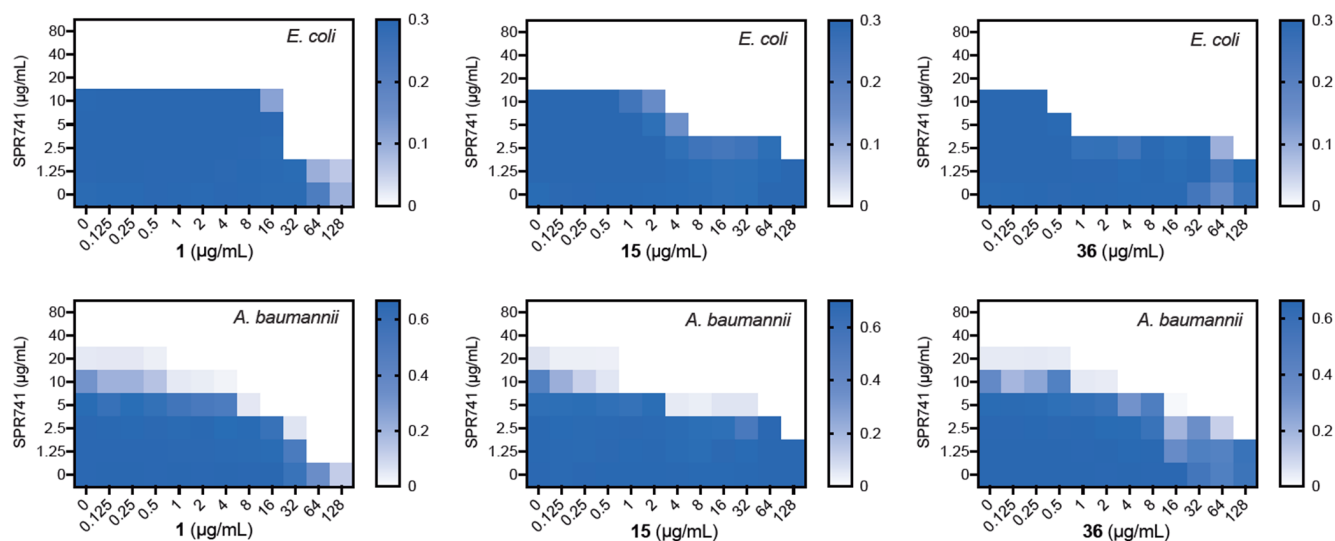
Figure 5. Checkerboard broth microdilution assays showing dose-dependent potentiation of metergoline (**1**) and analogues **15** and **38** by the OM permeabilizer SPR741 against wild-type strains of *Escherichia coli* MG1655 and *Acinetobacter baumannii* C0015 in MHB medium.

against additional Gram-negative bacteria. The compounds were inactive against wild-type strains of enterohemorrhagic *E. coli* (EHEC), *Acinetobacter baumannii*, *Burkholderia cenocepacia*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Table 3), as they were against the WT strains of *S.* Tm and *E. coli* MG1655 (Tables 1 and 2); however, since most analogues showed improved activities against efflux-deficient *S.* Tm ($\Delta tolC$) and hyperpermeable *E. coli* ($\Delta tolC$-pore), we reasoned that combination with an OM permeabilizer could also be used to improve the activity.

Compounds that physically disrupt the integrity of the OM (*e.g.*, polymyxins, pentamidine, peptide S25) can be used as adjuvants to sensitize Gram-negative bacteria to otherwise-inactive antimicrobials.[6,41−44] SPR741 (NAB741) is a poly-myxin analogue that has reduced antimicrobial potency and renal toxicity but retains its ability to disrupt the OM and is able to potentiate a range of antibiotics against Gram-negative bacteria.[25,45,46] Here we found that SPR741 was also successful in potentiating metergoline and analogues **15**, **28**, **38**, and **44** against Gram-negative bacteria (Table 3). For example, in the presence of 10 μg/mL SPR741, the MICs for 3-CF$_3$ derivative **28** were reduced >128-, >64-, >32-, and >128-fold against WT *E. coli*, EHEC, *A. baumannii*, and *B. cenocepacia*, respectively. Checkerboard broth microdilution assays with *E. coli* and *A. baumannii* confirmed that the interactions of SPR741 with metergoline, **15**, and **38** are synergistic (Figure 5). SPR741 is known to potentiate poorly against *K. pneumoniae* and *P. aeruginosa*,[45] however, and the MICs for the metergoline analogues were reduced only modestly (up to 8-fold) against *K. pneumoniae*.

CONCLUSIONS

Metergoline was discovered in a screen for inhibitors of *Salmonella* Typhimurium growth and was efficacious *in vivo* despite having weak antibiotic activity under standard *in vitro* assay conditions. As ergot alkaloids have not been studied systematically as antibacterials, we viewed metergoline as a new lead for antibiotic discovery. We synthesized a series of metergoline analogues that showed improved potency against Gram-positive bacteria and mycobacteria, and compound **38**

was also efficacious against MRSA in a murine skin infection model. Although metergoline analogues were inactive against WT strains of Gram-negative bacteria, they showed improved activity against efflux-deficient or hyperpermeable strains. The potency was also greatly improved with the OM permeabilizer SPR741, supporting the hypothesis that metergoline analogues have the potential to be developed into broad-spectrum antibiotics with improved permeability through Gram-negative outer membranes.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00648.

> Experimental procedures for MIC determination, disc diffusion assays, animal skin infection models, checker-board broth microdilution assays, synthetic experimental procedures, and NMR spectra (^{1}H, ^{13}C) for compounds synthesized (PDF)

> FAIR data, including the primary NMR FID files, for compounds **16−51** (ZIP)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

EHEC, enterohemorrhagic *Escherichia coli*; HWE, Horner−Wadsworth−Emmons; LPM, low-phosphate low-magnesium medium; MHB, Mueller−Hinton broth; MOPS, Neidhardt minimal medium for enterobacteria; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; OM, outer membrane; SCV, *Salmonella*-containing vacuole; WT, wild-type.

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